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The growth of fungal mycelium in forest soil layers*

BY

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INTRODUCTION

The determination of the quantity of living mycelium which is physiologically active in the soil should provide an index of fungal activity (PARKINSON, BALASOORIYA and WINTERHALDER, 1968). JONES and MOLLISON (1948) pointed out that there was some evidence that unstained hyphae in stained films of soil in agar represent dead hyphae. Such a distinction between stained and unstained hyphae was made by LATTER, CRAGG and HEAL (1967) who showed that estimations of the total length of mycelium are of limited value. They found a positive relationship between the amount of stained (live) hyphae and the overall activity of soil organisms in four types of moorland soils measured by respiration.

Up to now it has not been possible to measure fungal respiration without, at the same time, including the respiration of all other micro-organisms present. An objection against taking mycelium as an index for fungal activity is its variable susceptibility to decomposition by mycolytic organisms (NAGEL-DE BOOIS, 1971). For this reason estimation of fungal growth gives a better index of fungal activity in soil. Only relative methods are available at present for measurement of fungal growth (CHOLODNEY, 1930; WAID and WOODMAN, 1957). NAGEL-DE BOOIS (1971) made an attempt to quantify growth estimations obtained with the Waid and Woodman nylon-gauze technique.

During 1967, 1968 and 1969 monthly observations were made as a part of the I.B.P. (International Biological Programme) study on production and decomposition of organic matter in oak woodlands. Seasonal variations in the amount of mycelium and in growth rate can give an indication of the main factors affecting fungal activity in soil. In our opinion counts of live and dead mycelium using the Jones and Mollison agar-slide technique can only provide part of this information. We therefore also used the nylon-gauze method of Waid and Woodman for measurement of fungal growth.

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MATERIAL AND METHODS

LOCATION: The site under study, Meerdink, is a 135-year-old wood of Quercus petraea mixed with Fagus silvatica, covering an area of 7 ha. situated 6 km south of Winterswijk in the eastern part of the Netherlands. Undergrowth of Hedera helix, Rubus sp. and Pteridium aquilinum covers the soil locally. To make the experiments as simple as possible an area without undergrowth was selected for the mycological work. This site was classified by the Soil Survey Institute, Wageningen, as having a « goor » earth soil. BAKKER and SCHELLING (1966) define such a soil as a « sandy hydroearth soil without rusty mottles, or, when mottles are present they begin at a depth of more than 35 cm ».

Monthly samples were taken from the horizons as specified below:

	pH-KC_l	loss on ignition	organic Carbon
L : 80 % oak litter, 20 % beech litter	3.2	93.7 %	43.1 %
F : fragmentation layer, 1.5 cm thick	3.0	73.5 %	34.8 %
H : upper 2 cm of the ± 10 cm thick humus layer ..	2.8	53.1 %	25.8 %
A_l : upper 2 cm of mineral horizon	2.8	6.5 %	3.4 %

SAMPLING: For each horizon ten small samples were taken from each of the four horizons and bulked to one sample per horizon. The samples of the F, H and A_l horizons were taken from a drill-core (Ø 5 cm). The litter of the L horizon was sampled by hand.

MOISTURE CONTENT: Determined by drying at 105° C. The average moisture content of the monthly samples was:

L : 53 % (varying from 8 — 80 %)
F : 70 % (varying from 51 — 76 %)
H : 66 % (varying from 55 — 75 %)
A_l : 23 % (varying from 15 — 37 %).

The moisture content varies most in the L horizon; in summer leaves can be completely dry. This never occurs in the other horizons.

pH: Determined in a suspension of 1 volume soil in 2 volumes KC_l, 1 N.

ORGANIC MATTER: Loss of ignition corresponds fairly well with total amount of organic matter present. The content of iron concretions is low, so their water retention at ignition is negligible.

ORGANIC CARBON: Determination by dry combustion at 500° C.

LENGTH OF FUNGAL MYCELIUM: The agar-slide technique of JONES and MOLLISON (1948) was used, this being the best technique according to NICHOLAS and PARKINSON (1967). Fixed quantities of the samples (L, 2 grams; F, 5 grams; H, 2.5 grams and A_l, 5 grams) were mixed with 25 ml water for 1 minute in a homogenizer at 30.000 rpm. In this way mycelium inside the leaf litter was also revealed. Slides were prepared from 5 ml of these solutions in 10 ml liquid 1.5 % water agar. Measurements of mycelium content started in January 1967. Mycelial counts of stained and unstained hyphae started in January 1968.

FUNGAL GROWTH: A somewhat modified WAID and WOODMAN method (1957) was used. 3 Nylon nets 10 cm wide and 15 cm long with 125 meshes per cm were

buried vertically in the soil for a period of three weeks. 600 Meshes were examined from each horizon for the presence of fungal mycelium after exposure. Fungal growth is expressed as the percentage of meshes with mycelium.

TEMPERATURE: Air temperature and temperatures at soil depths of 5 and 10 cm were registered with a thermograph (Wilh. Lambrecht KG 257a). The average weekly temperatures of the litter layer to a depth of 5 cm are shown in Figure 1.

PRECIPITATION: Rainfall in the open was measured by a pluviometer by the Hellman method (Wilh. Lambrecht KG 1507).

The weekly rainfall is recorded in Figure 1.

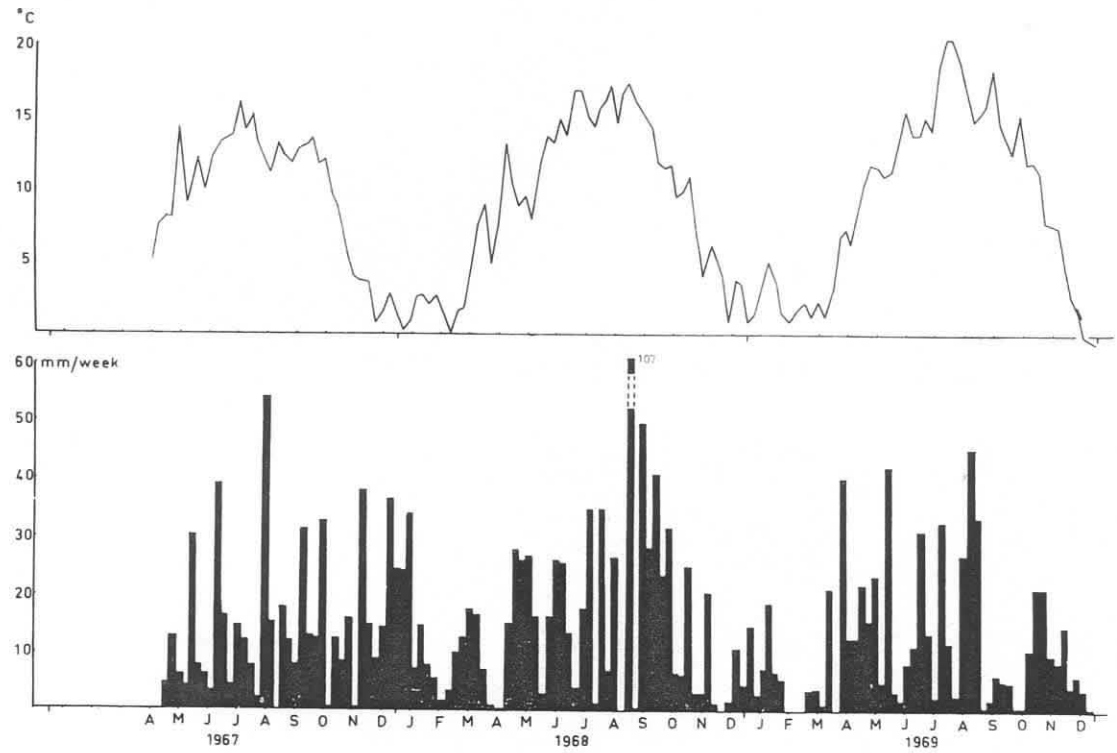

FIG. 1. — Soil temperature at a depth of 5 cm, and precipitation.

RESULTS

Mycelial growth.

Growth of fungal mycelium on nylon gauze as measured monthly for three years, is presented in Fig. 2 (L horizon), Fig. 3 (F horizon), Fig. 4 (H horizon) and Fig. 5 (A_1 horizon). The graphs and Table I show that mycelial growth in the L and F horizons takes place at the same rate. Seasonal changes in the growth rate of mycelium are also synchronous in the upper layers. Peaks in mycelium growth occur in spring/early summer and autumn. Low mycelial growth is recorded mostly during February, March and April, but also in the late summer during August and September. This corresponds with earlier results from another oak forest (WITKAMP, 1960; NAGEL-DE BOOIS and JANSEN, 1967).

Mycelial growth in the H and A_1 horizons is low compared with growth

in the upper litter layers. Variations in fungal growth during the year are less significant. But a high rate of growth in these horizons corresponds in many cases with growth in the F horizon. Fig. 5 does not show greater fungal growth in the A_1 horizon during autumn. Note the low rate of growth of fungal mycelium in 1968 which is half the growth rate during 1967 and 1969 in the H and A_1 horizons. In the L and F horizons mycelial growth is also less for 1968, but less significantly so.

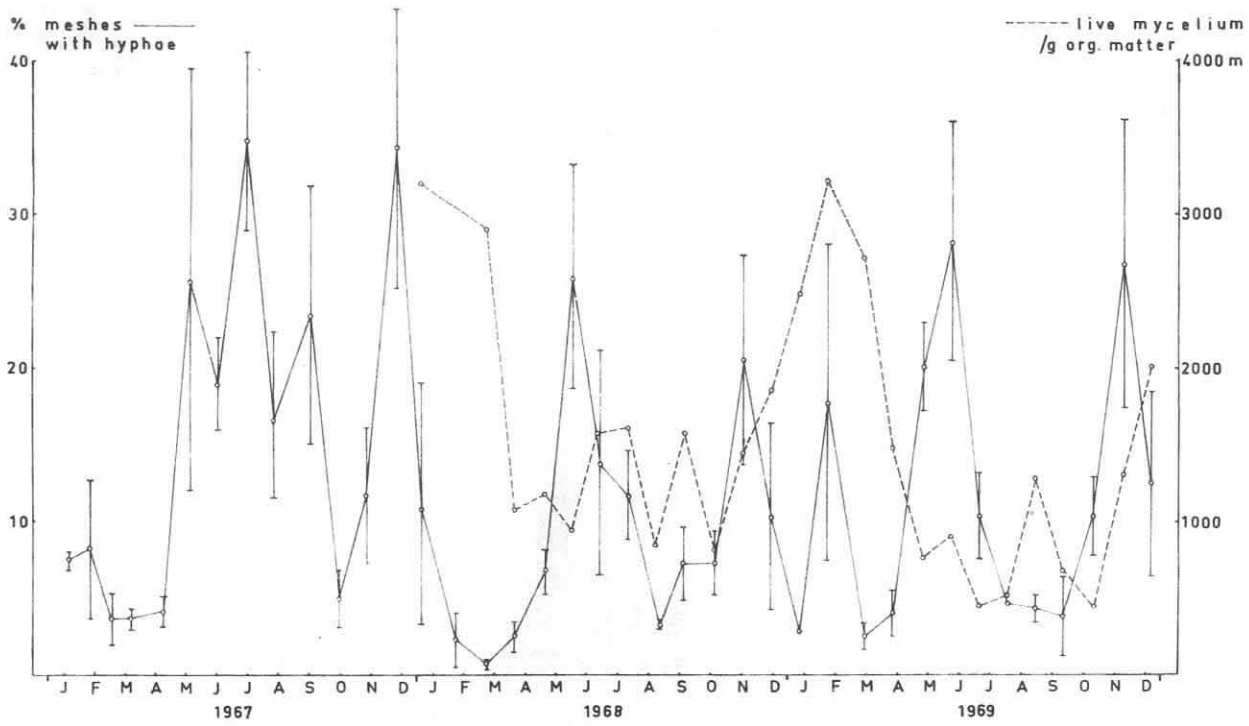

FIG. 2. — Mycelium growth on nylon gauze and amount of live mycelium in the L horizon.

Quantity of fungal mycelium.

The length of fungal mycelium is expressed per gram of organic matter to provide a means of comparing litter and soil layers. Table I also shows the amount of fungal mycelium per gram dry material to facilitate comparison with other publications.

The figures for the total mycelium content (Fig. 6) show a discrepancy between the L and F horizons, with a low level of mycelium, and the H and A_1 horizons with an amount of fungal mycelium which is four times higher.

In the deeper soil and litter layers most mycelium is dead, as is shown in Table I. The amount of living mycelium in the L horizon is much higher than in the other three horizons. During the first six months after litter fall 90 % of the mycelium on the newly fallen litter is live. After a year only half of the amount of mycelium on leaf litter is live.

Seasonal variations in mycelial growth are not reflected in the amount of fungal mycelium present, neither in the total amount nor in the amount of living mycelium. NICHOLAS and others (1965) found a sustained increase in

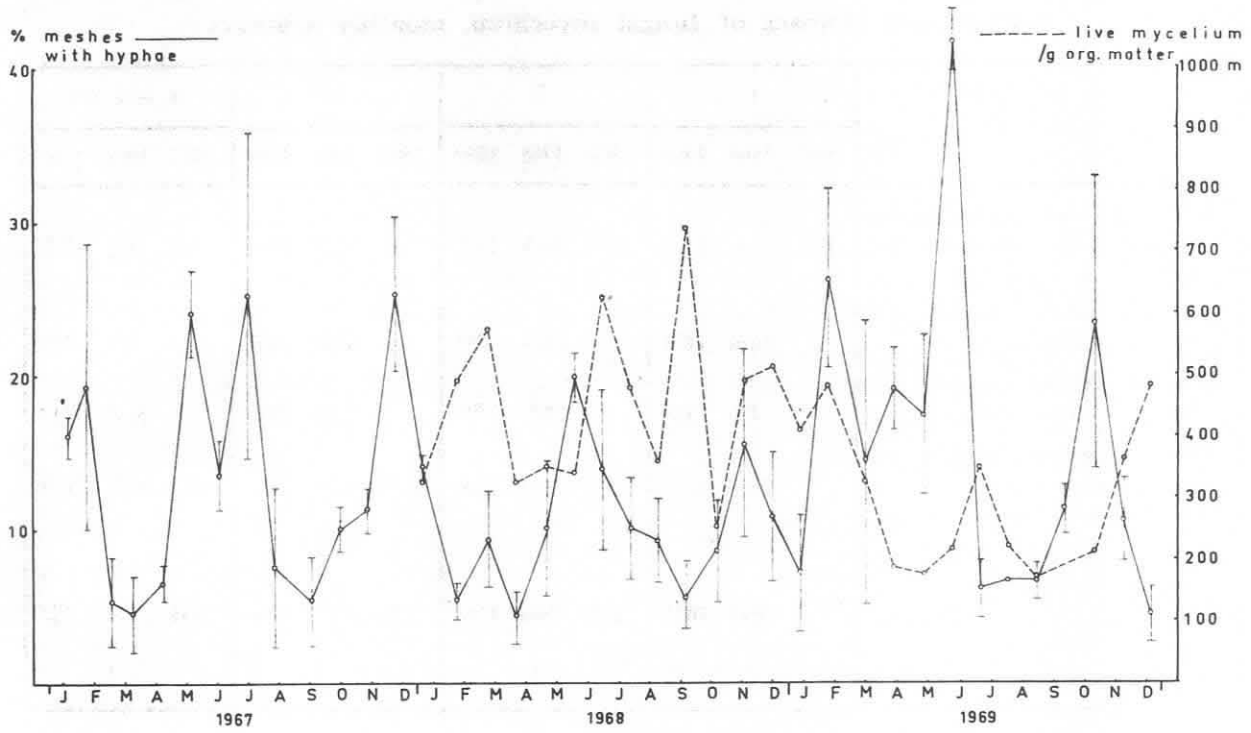

FIG. 3. — Mycelium growth on nylon gauze and amount of live mycelium in the F horizon.

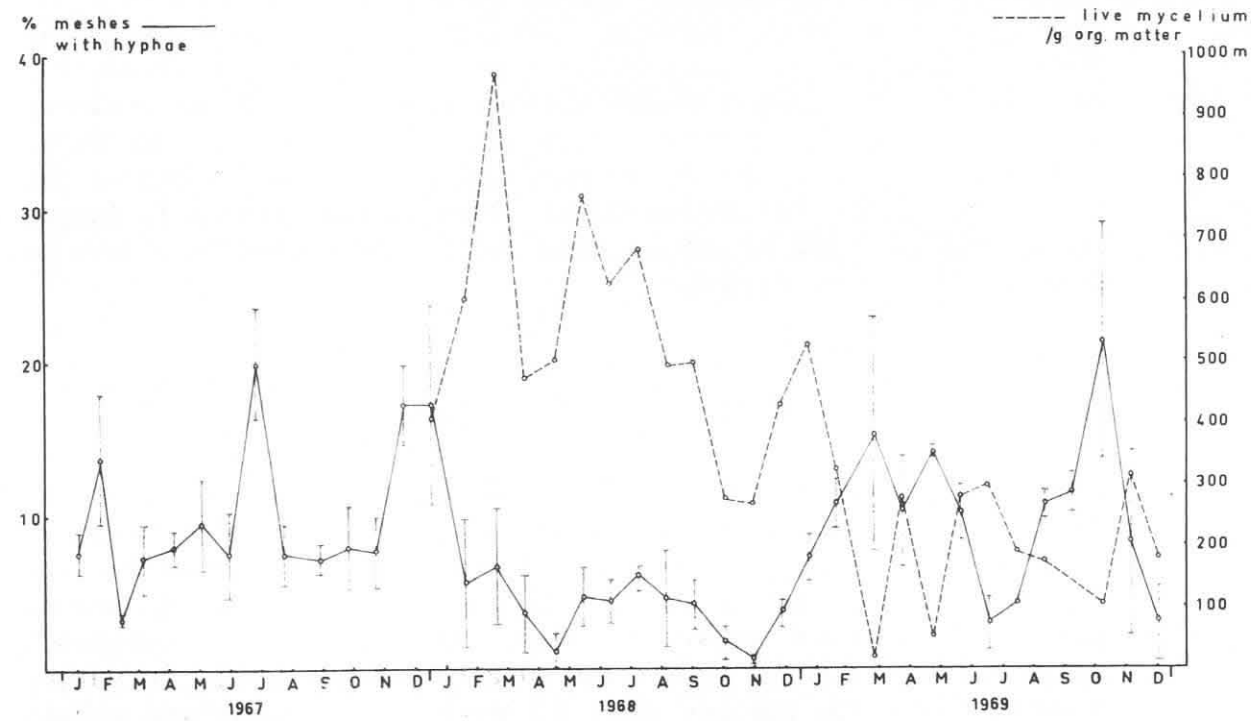

FIG. 4. — Mycelium growth on nylon gauze and amount of live mycelium in the H horizon.

TABLE I

Growth and biomass of fungal mycelium, monthly averages

	L			F			H			A_1 — 2 cm		
	1967	1968	1969	1967	1968	1969	1967	1968	1969	1967	1968	1969
Percentage of nylon meshes colonized by fungal hyphae.	15.2	9.4	11.2	13.4	10.5	14.9	9.6	5.0	10.0	7.0	3.2	7.5
Live mycelium, length (m) per g. organic matter		1694	1478		451	299		540	237		333	190
Dead mycelium, length (m) per g. organic matter		455	327		1829	1701		7933	7450		6538	7617
Total mycelium, length (m) per g. organic matter		2149	1705	2240	2280	2000	5253	8473	7687	5711	6871	7807
Total mycelium, length (m) per g. oven dry weight of soil		2014	1683	1617	1686	1503	2959	4279	3818	335	514	487
Percentage living mycelium ..		79	82		20	15		6	3		5	3

the production of fungal mycelium during the autumn-winter period within the A_0h, A_1 and A_2 horizons of Delamere forest while studying the amounts of fungal mycelium. They also indicated the possibility of an increase in mycelium production during early summer. In our investigations the same periods of fungal activity were recorded for the A_0h horizon by measuring fungal growth. The amounts of fungal mycelium gave no conclusive evidence of seasonal fluctuations. Nor did the estimations of live mycelium in the L, F and H horizons. Only in the A_1 horizon can a seasonal pattern in the amount of live mycelium be distinguished. This is not related to fungal growth. Perhaps these high mycelial counts were only caused by a lack of decomposition of the fungal hyphae.

DISCUSSION AND CONCLUSIONS

The value of the nylon mesh method for the measurement of the mycelial growth rate is open to doubt. The exposure period of three weeks is most arbitrary. This was shown by experiments with large quantities of pieces of nylon gauze exposed to the litter layers from which five specimens were removed and counted every two days. During summer maximum colonization was reached in 1-2 weeks, but at a rather low level. In winter maximum colonization was reached after 4-7 weeks. This maximum colonization of the nylon gauze represents a combination of mycelium growth and decomposition. Both are variable factors. During the first week of exposure to the soil there is hardly any decomposition of the mycelium on the nylon gauze (NAGEL-DE BOOIS, 1971).

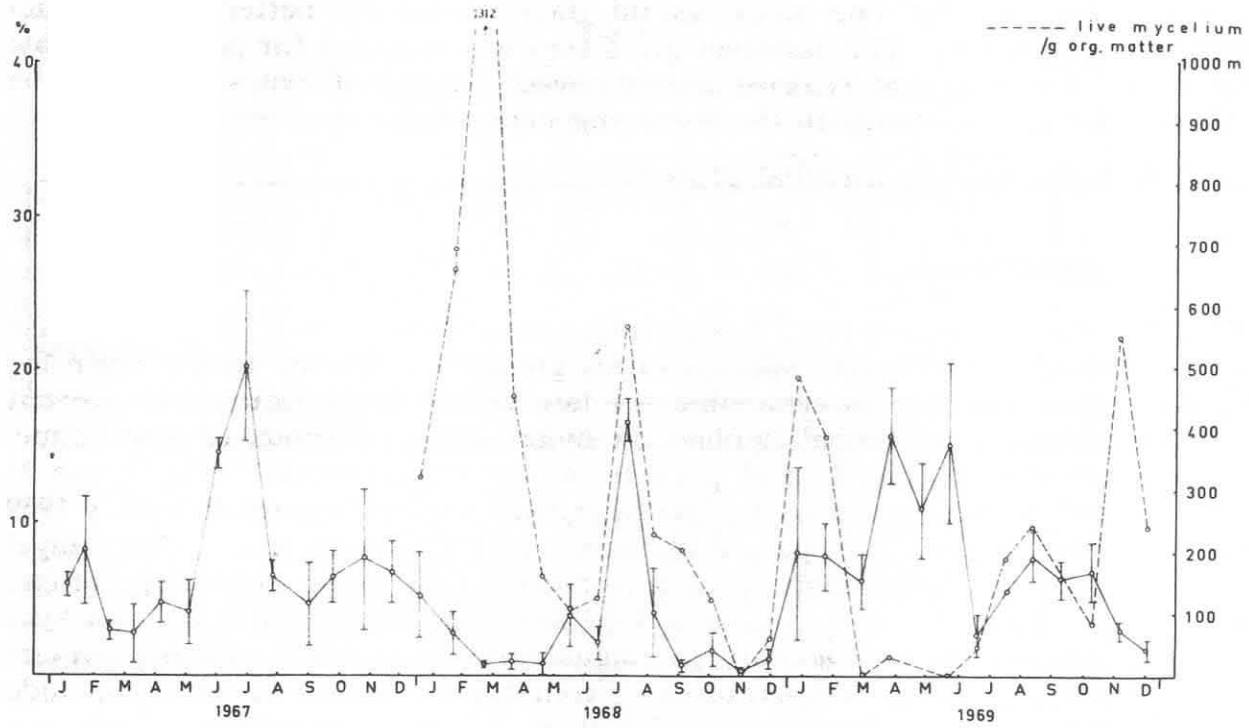

FIG. 5. — Mycelium growth on nylon gauze and amount of live mycelium in the A_1 horizon.

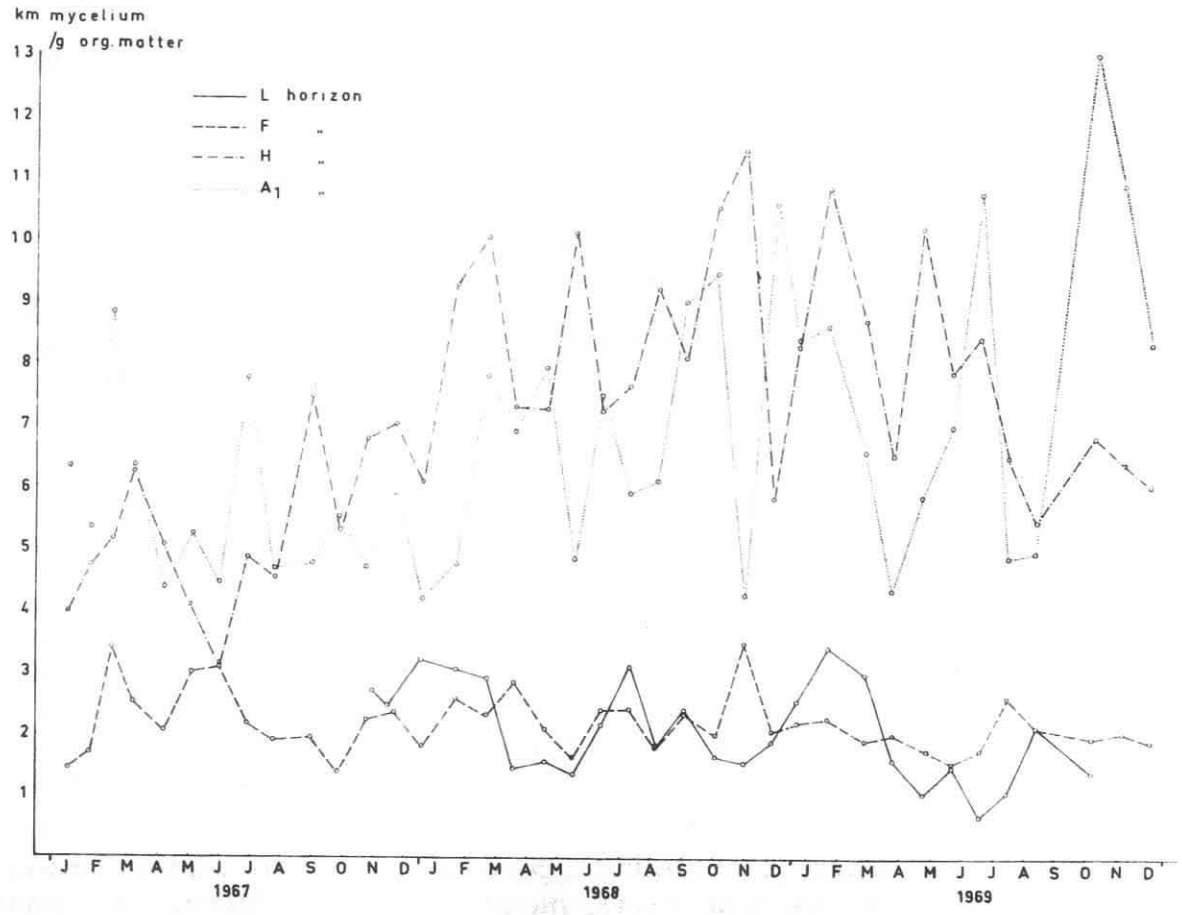

FIG. 6. — Total amount of mycelium in L, F, H and A_1 horizons.

An exposure of one week would theoretically be better for mycelial growth estimation. This however gives very low readings for periods of slow growth. For practical reasons a three week period of exposure would be better, as will be shown in the following paragraphs.

Factors affecting mycelial growth.

a. **Temperature.**

Soil fungi grow within a wide temperature range (WAID, 1960). Because the composition of fungal species varies greatly an optimal temperature for fungal growth is not ascertainable. A low winter temperature can prevent the development of fungal hyphae on nylon gauze. Periods of low temperatures in winter (Fig. 1) and periods of low fungal growth (Fig. 2 and 3) correspond to some extent. Increased growth during January/February 1969 was perhaps caused by a period of higher winter temperatures. But fungal growth was correspondingly slow in April then too. Also, soil temperatures were low during the first months of 1969 whilst mycelial growth was not low; and decreasing growth activity in August and September was not accompanied by decreasing temperatures. From these results we may conclude that there are other environmental factors besides temperature which can strongly influence fungal growth.

b. **Water table.**

In winter the ground water is 25-45 cm under the surface. The consequent low aeration of the soil could be a reason for the variations in mycelial growth in the A_1 horizon. But during periods with a constant high subsoil water level, as from the middle of December 1967 until March 1968 and from the middle of September 1968 until May 1969, there were fluctuations in mycelial growth in the A_1 horizon. During autumn 1969 the water level did not rise, nevertheless mycelial growth in the A_1 horizon did not increase as in the other horizons. It seems that mycelium growth is not affected by the water level.

c. **Moisture.**

In order to analyse the influence of moisture content on the growth of fungal mycelium in the litter layers, the moisture content of leaf litter should have been estimated more frequently than once a month.

As it is a comparison of weekly rainfall records (Fig. 1) and mycelial growth appears to be preferable. Periods of rainfall sufficient to maintain a high moisture content in the litter often correlate with periods of high fungal growth. But lack of fungal growth during late summer cannot generally be explained by lack of rain. Poor mycelial growth in 1968 was not caused by the absence of precipitation (Table II).

PARKINSON and others (1968) found a good measure of correlation between respiratory activity of the soil layers, measured at 25° C during the whole year, and soil moisture content.

TABLE II

Quarterly rainfall in mm. in the open

	1967	1968	1969
January - March	188*	183	67
April - June	182*	192	212
July - September	200	330	207
October - December	213	174	100

* Figures from K.N.M.I. (Royal Dutch Meteorological Institute).

In the F, H and A_1 horizons of the Meerdink forest moisture content is seldom low for long periods. Fig. 7 illustrates low fungal growth during the first days of exposure of the nylon nets to the dry litter layers. After this period an equilibrium between mycelial growth and decomposition is reached with a fairly high level of colonization of the nylon gauze. The average soil

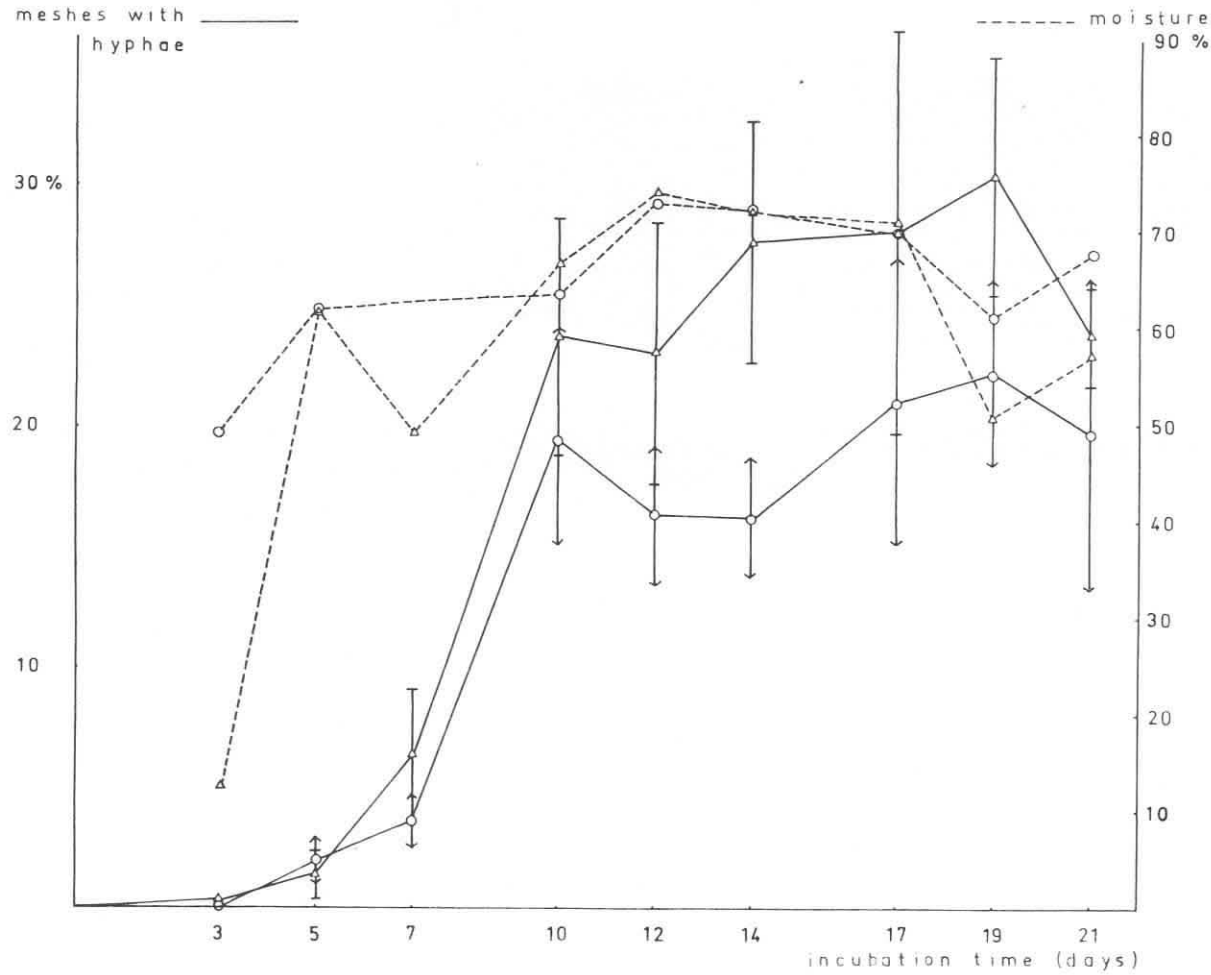

FIG. 7. — Hyphal colonization of nylon gauze in the L (——Δ——) and F horizon (——o——) in relation to the moisture content of L (— —Δ— —) and F horizon (— —o— —). Exposure from 19-6-1970 to 10-7-1970.

temperature was 15.4° C (var. 11°-18° C). In Fig. 8 the same situation is presented with dry litter surrounding the nylon gauze during the first days of exposure to the soil. After a rise in the moisture content of the litter, colonization by fungi soon reached a maximum at a low level. The average soil temperature during the period of observation was 13.2° C (var. 11°-17° C). The difference between these two examples stems from the season in which

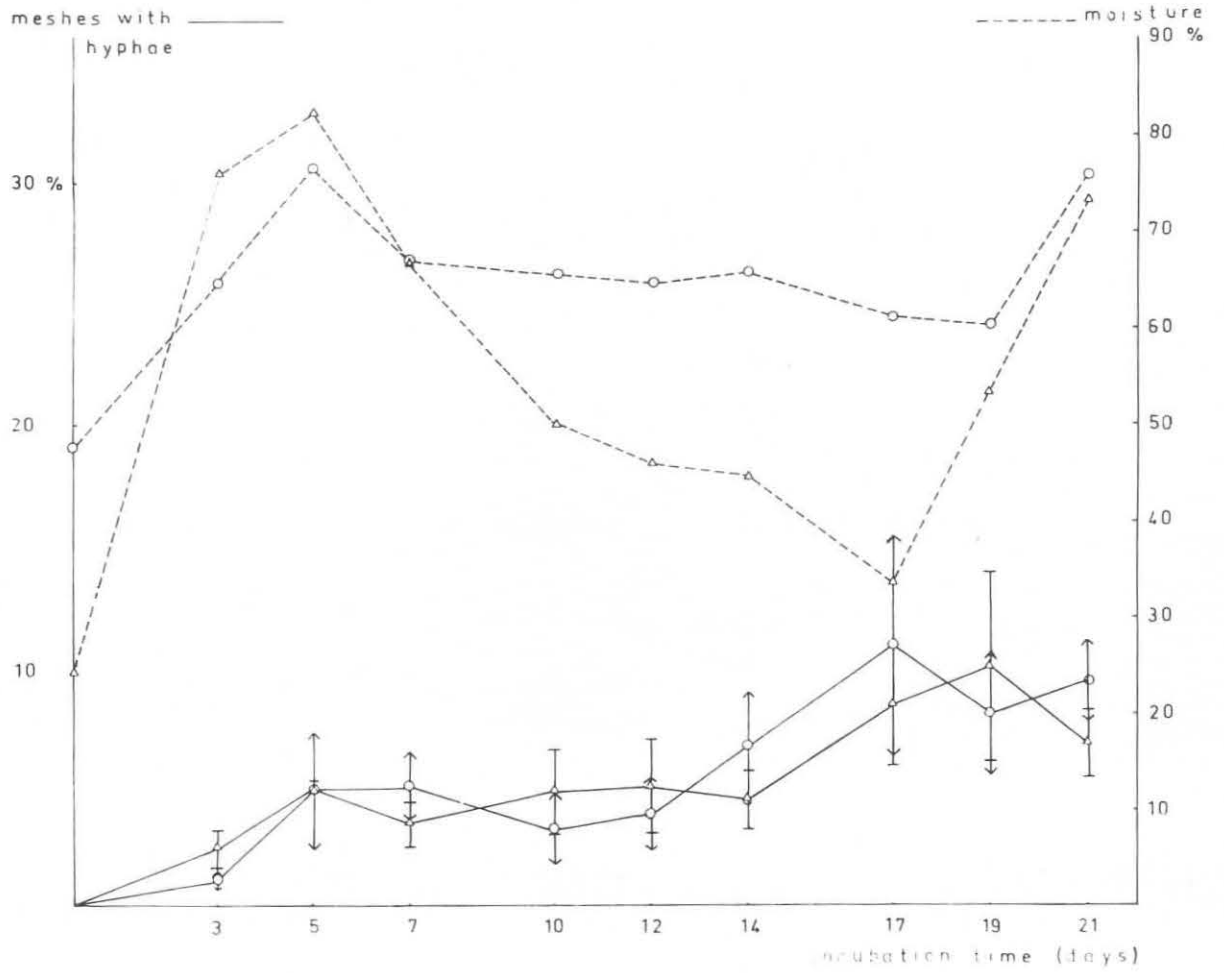

FIG. 8. — Hyphal colonization of nylon gauze in the L (——Δ——) and F horizon (——o——) in relation to the moisture content of L (— —Δ— —) and F horizon (— —o— —). Exposure from 11-9-1970 to 2-10-1970.

the observations were made. Fig. 7 illustrates a colonization pattern in a period of high fungal activity (early summer). Fig. 8 shows the colonization in a period of low fungal growth. From these examples it is clear that three weeks exposure of the nylon gauze to the soil gives an indication of the maximum colonization, which is not always the case after one or two weeks. So, we may conclude, fungal growth is influenced by the moisture content of the soil. But seasonal variations in mycelial growth cannot be explained in this way.

d. **Nutrient supplies.**

The increased growth of mycelium in the L, F and H horizons after litter fall points to stimulation by new added nutrients. Possibly these nutrients do not penetrate the A_1 horizon where no increase in the autumn growth of fungi was recorded.

In general, low mycelial growth during summer may be caused by a lack of nutrients available for decomposition. Periods of high growth in autumn and spring/early summer can be regarded as periods during which sufficient nutrients are present in the soil. Only low temperatures prevent fungal growth during winter.

NICHOLAS a.o. (1965) concluded that litter fall might be the main contributory factor of the increase in the production of mycelium in the different A horizons. In fact they measured the amount of mycelium present at one moment in time, and thus measured a result of two simultaneous processes, growth and decomposition. Our estimations of the amount of fungal mycelium in the L, F and H horizons provide less support for the theory of the influence of leaf fall than growth estimations do.

SUMMARY

The growth of fungal mycelium on nylon gauze shows periods of high fungal activity in spring/early summer and in autumn in the L, F and H horizons. The A_1 horizon shows a less pronounced pattern. The amount of living or of all mycelium present is influenced by the growth of mycelium and by decomposition. Therefore the seasonal variations shown by monthly estimations of the live mycelium content of soil are not so pronounced.

Growth of mycelium is partly determined by climatic conditions. Only in the L horizon did long periods of drought prevent fungal growth. But a low rate of fungal growth during the summer was probably caused by the lack of rapidly decomposing nutrients.

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